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(54) Title: RNA RESPIRATORY SYNCYTIAL VIRUS VACCINES

(57) Abstract

A vector comprising a first DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of complete alphavirus DNA genome replication regions, a second DNA sequence encoding a paramyxovirus protein, particularly a respiratory syncytial virus fusion (RSV F) protein or an RSV F protein fragment that generates antibodies that specifically react with RSV F protein, the first and second DNA sequences being under the transcriptional control of a promoter is described. Such vector may be used to produce an RNA transcript which may be used to immunize a host, including a human host, to protect the host against disease caused by paramyxovirus, particularly respiratory syncytial virus, by administration to the host. The RNA transcript may be formed by linearization of the vector through cleavage at a unique restriction site in a plasmid vector and then transcribing the linear molecule.

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TITLE OF INVENTIONRNA RESPIRATORY SYNCYTIAL VIRUS VACCINESFIELD OF INVENTION

5 The present invention relates to the field of paramyxoviridae vaccines and is particularly concerned with vaccines comprising RNA encoding the fusion (F) protein of respiratory syncytial virus (RSV).

BACKGROUND OF THE INVENTION

10 Human respiratory syncytial virus (RSV) has been identified as a major pathogen responsible for severe respiratory tract infections in infants, young children and the institutionalized elderly (refs. 1,2,3,4 - throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby 20 incorporated by reference into the present disclosure). Global mortality and morbidity figures indicate that there is an urgent need for an efficacious RSV vaccine (refs. 5,6). In the USA alone, approximately 100,000 children are hospitalized annually with severe cases of 25 pneumonia and bronchiolitis resulting from an RSV infection. Inpatient and ambulatory care for children with RSV infections has been estimated to cost in excess of \$340 million each year in the USA. The World Health Organization (WHO) and the National Institute of Allergy 30 and Infectious Disease (NIAID) vaccine advisory committees have ranked RSV second only to HIV for vaccine development. Both the annual morbidity and mortality figures as well as the staggering health care costs for managing RSV infections have provided the 35 incentive for aggressively pursuing the development of

efficacious RSV vaccines. However, such a vaccine is still not available.

Formalin-inactivated (FI-RSV) and live attenuated RSV vaccines have failed to demonstrate efficacy in 5 clinical trials (refs. 7,8,9,10). Moreover, the formalin-inactivated RSV vaccine caused enhanced disease in some children following exposure to wild-type RSV (refs. 7,8,9,10). Elucidation of the mechanism(s) involved in the potentiation of RSV disease is important 10 for the design of safe RSV vaccines, especially for the seronegative population. Recent experimental evidence suggests that an imbalance in cell-mediated responses may contribute to immunopotentiation. Enhanced histopathology observed in mice that were immunized with 15 the FI-RSV and challenged with virus could be abrogated by depletion of CD4+ cells or both interleukin-4 (IL-4) and IL-10.

The RSV fusion (F) glycoprotein is one of the major 20 immunogenic proteins of the virus. This envelope glycoprotein mediates both fusion of the virus to the host cell membrane and cell-to-cell spread of the virus (ref. 1). The F protein is synthesized as a precursor (F₀) molecule which is proteolytically cleaved to form a disulphide-linked dimer composed of the N-terminal F₂ 25 and C-terminal F₁ moieties (ref. 11). The amino acid sequence of the F protein is highly conserved among RSV subgroups A and B and is a cross-protective antigen (refs. 6,12). In the baculovirus expression system, a truncated secreted version of the RSV F protein has been 30 expressed in *Trichoplusia ni* insect cells (ref. 13). The recombinant protein was demonstrated to be protective in the cotton rats (ref. 13).

Studies on the development of live viral vaccines 35 and glycoprotein subunit vaccines against parainfluenza virus infection are being pursued. Clinical trial results with a formalin-inactivated PIV types 1,2,3

vaccine demonstrated that this vaccine was not efficacious (refs. 14, 15, 16). Further development of chemically-inactivated vaccines was discontinued after clinical trials with a formalin-inactivated RSV vaccine
5 demonstrated that not only was the vaccine not effective in preventing RSV infection but many of the vaccinees who later became infected with RSV suffered a more serious disease. Most of parainfluenza vaccine research has focussed on candidate PIV-3 vaccines (ref. 17) with
10 significantly less work being reported for PIV-1 and PIV-2. Recent approaches to PIV-3 vaccines have included the use of the closely related bovine parainfluenza virus type 3 and the generation of attenuated viruses by cold-adaptation of the virus (refs. 18, 19, 20, 21).

15 Another approach to parainfluenza virus type 3 vaccine development is a subunit approach focusing on the surface glycoproteins hemagglutinin-neuraminidase (HN) and the fusion (F) protein (refs. 22, 23, 24). The HN antigen, a typical type II glycoprotein, exhibits
20 both haemagglutination and neuraminidase activities and is responsible for the attachment of the virus to sialic acid containing host cell receptors. The type I F glycoprotein mediates fusion of the viral envelope with the cell membrane as well as cell to cell spread of the
25 virus. It has recently been demonstrated that both the HN and F glycoproteins are required for membrane fusion. The F glycoprotein is synthesized as an inactive precursor (F) which is proteolytically cleaved into disulfide-linked F2 and F1 moieties. While the HN and F
30 proteins of PIV-1, -2 and -3 are structurally similar, they are antigenically distinct. Neutralizing antibodies against the HN and F proteins of one of PIV type are not cross-protective. Thus, an effective PIV subunit vaccine must contain the HN and F glycoproteins
35 from the three different types of parainfluenza viruses. Antibody to either glycoprotein is neutralizing in

vitro. A direct correlation has been observed between the level of neutralizing antibody titres and resistance to PIV-3 infections in infants. Native subunit vaccines for parainfluenza virus type 3 have investigated the 5 protectiveness of the two surface glycoproteins. Typically, the glycoproteins are extracted from virus using non-ionic detergents and further purified using lectin affinity or immunoaffinity chromatographic methods. However, neither of these techniques may be 10 entirely suitable for large scale production of vaccines under all circumstances. In small animal protection models (hamsters and cotton rats), immunization with the glycoproteins was demonstrated to prevent infection with live PIV-3 (refs. 25, 26, 27, 28, 29). The HN and F 15 glycoproteins of PIV-3 have also been produced using recombinant DNA technology. HN and F glycoproteins have been produced in insect cells using the baculovirus expression system and by use of vaccinia virus and adenovirus recombinants (refs. 30, 31, 32, 33, 34). In 20 the baculovirus expression system, both full-length and truncated forms of the PIV-3 glycoproteins as well as a chimeric F-HN fusion protein have been expressed. The recombinant proteins have been demonstrated to be protective in small animal models (see WO91/00104, US 25 Application No. 07/773,949 filed November 29, 1991, assigned to the assignee hereof).

Semliki Forest virus (SFV) is a member of the Alphavirus genus in the Togaviridae family. The mature virus particle contains a single copy of a ssRNA genome 30 with a positive polarity that is 5'-capped and 3'-polyadenylated. It functions as an mRNA and naked RNA can start an infection when introduced into cells. Upon infection/transfection, the 5' two-thirds of the genome 35 is translated into a polyprotein that is processed into the four nonstructural proteins (nsP1 to 4) by self cleavage. Once the ns proteins have been synthesized

they are responsible for replicating the plus-strand (42S) genome into full-length minus strands (ref. 35). These minus-strands then serve as templates for the synthesis of new plus-strand (42S) genomes and the 26S 5 subgenomic mRNA (ref. 35). This subgenomic mRNA, which is colinear with the last one-third of the genome, encodes the SFV structural proteins. In 1991 Liljestrom and Garoff (ref. 36) designed a series of expression vectors based on the SFV cDNA replicon. These 10 alphavirus vectors also are described in WO 92/10578, the disclosure of which is incorporated herein by reference. These vectors had the virus structural protein genes deleted to make the way for heterologous inserts, but preserved the nonstructural coding region 15 for production of the nsP1 to 4 replicase complex. Short 5' and 3' sequence elements required for RNA replication were also preserved. A polylinker site was inserted downstream from the 26S promoter followed by translation stop sites in all three frames. An SpeI site was 20 inserted just after the 3' end of the SFV cDNA for linearization of the plasmid for use *in vitro* transcription reactions.

Injections of SFV RNA encoding a heterologous protein have been shown to result in the expression of 25 the foreign protein and the induction of antibody in a number of studies (refs. 37, 38). The use of SFV RNA inoculation to express foreign proteins for the purpose of immunization would have several of the advantages associated with plasmid DNA immunization. For example, 30 SFV RNA encoding a viral antigen may be introduced in the presence of antibody to that virus without a loss in potency due to neutralization by antibodies to the virus. Also, because the protein is expressed *in vivo* the protein should have the same conformation as the 35 protein expressed by the virus itself. Therefore, concerns about conformational changes which could occur

during protein purification leading to a loss in immunogenecity, protective epitopes and possibly immunopotentiation, could be avoided by nucleic acid immunization.

5 Immunization with SFV RNA also has several unique advantages over plasmid DNA immunization. SFV is one of the most efficiently replicating viruses known. After a few hours, up to 200,000 copies of the plus-RNAs can be made in a single cell. These SFV RNAs are so abundant
10 almost all of the cells ribosomes are enrolled in the synthesis of the SFV encoded proteins, thus overtaking host cell protein synthesis (ref. 36). Therefore, it should require a smaller dose of SFV RNA and less time to achieve a protective effect as compared to plasmid
15 DNA immunization. Secondly, RNA, unlike DNA, poses no potential threat of integrating into the cell genome. Thirdly, SFV RNA replication and expression occurs only in the cytoplasm of the cell. Therefore, problems involving nuclear transport and splicing associated with
20 nucleus-based expression systems (DNA immunization) are absent. Fourthly, since the replication of the SFV RNA is transient and RNA is quite labile, the SFV RNA will not persist for long periods after immunization like DNA plasmids.

25 In WO 95/27044, the disclosure of which is incorporated herein by reference, there is described the use of alphavirus cDNA vectors based on cDNA complementary to the alphavirus RNA sequence. Once transcribed from the cDNA under transcriptional control
30 of a heterologous promoter, the alphavirus RNA is able to self-replicate by means of its own replicase and thereby amplify the copy number of the transcribed recombinant RNA molecules.

35 In copending US Patent Application No. 08/476,397 filed June 7, 1995, (WO 96/40945), assigned to the assignee hereof and the disclosure of which is

incorporated herein by reference, there are described certain plasmid constructs used for DNA immunization which include forms of the RSV F gene. As seen therein, one plasmid pXL2 conferred complete protection on mice
5 to challenge by live RSV when administered intranasally. This plasmid contains a gene encoding a truncated RSV F protein lacking the transmembrane portion of the protein, the immediate-early promoter enhancer and intron sequences of human cytomegalovirus (CMV) and the
10 intron II sequences of rabbit β -globin to prevent aberrant splicing. The same plasmid construct but without the intron II sequences of rabbit β -globin, i.e. pXL1, provided only partial protection. Similarly, plasmid construct pXL4, which is the same as pXL2 except
15 the RSV F gene encodes the full length RSV protein, provided partial protection while the corresponding construct lacking the intron II sequence of rabbit β -globin, i.e. pXL3, conferred no protection.

These data show that the absence of elements to
20 reduce aberrant splicing adversely affects the protective ability of the plasmid. Aberrant splicing occurs during nuclear transcription of DNA to RNA. By employing RNA transcripts for immunization, the need for nuclear processing is avoided and aberrant splicing is
25 unable to occur. This enables the use of the intron II sequences from non-human sources to be avoided.

The use of RNA transcripts for administration to the host enables there to be obtained total protection to challenge using a lower dose in less time than when
30 employing the DNA plasmids described in USAN 08/476,397 (WO 96/40945). The use of the RNA transcripts avoids persistence of DNA in the immunized host and potential integration.

The ability to immunize against disease caused by
35 RSV by immunization with naked SFV RNA encoding the RSV F protein, particularly the secreted version of the RSV

F protein, was unknown before the present invention and could not be predicted on the basis of the known prior art. Infection with RSV leads to serious disease. It would be useful and desirable to provide improved 5 vectors for *in vivo* administration of immunogenic preparations, including vaccines, for protection against disease caused by RSV. In particular, it would be desirable to provide vaccines that are immunogenic and protective in the elderly and paediatric human 10 populations, including seronegative infants, that do not cause disease enhancement (immunopotentiation).

SUMMARY OF THE INVENTION

The present invention provides novel immunogenic materials and immunization procedures based on such 15 novel materials for immunizing against disease caused by paramyxoviridae, including respiratory syncytial virus and parainfluenza virus. In particular, the present invention is directed towards the provision of RNA 20 vaccines against disease caused by infection with paramyxoviridae.

In accordance with one aspect of the present invention, there is provided a vector, comprising a first DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the 25 complement of complete alphavirus RNA genome replication regions; a second DNA sequence encoding a paramyxovirus protein or a protein fragment that generates antibodies that specifically react with the paramyxovirus protein; the second DNA sequence being 30 inserted into a region of the first DNA sequence which is non-essential for replication; the first and second DNA sequences being under transcriptional control of a promoter.

The paramyxovirus protein may be selected from the 35 group consisting of a parainfluenza virus (PIV) and a respiratory syncytial virus (RSV). The PIV protein may

be PIV-1, PIV-2, PIV-3 or PIV-4, particularly the HN or F glycoproteins of PIV-3. The RSV protein particularly may be the F or G glycoprotein of RSV.

5 The second DNA sequence may encode a full length RSV F protein, or may encode a RSV F protein lacking the transmembrane anchor and cytoplasmic tail. The lack of the coding region for the transmembrane anchor and cytoplasmic tail results in a secreted form of the RSV F protein.

10 The second DNA sequence preferably encodes a RSV F protein and lacks a SpeI restriction site, and optionally, also lacking the transmembrane anchor and cytoplasmic tail encoding region. The absence of the SpeI restriction site may be carried out by mutating 15 nucleotide 194 (T) of the RSV F gene to a C, which eliminates the SpeI without altering the amino acid sequence. The nucleotide sequence (SEQ ID No: 1) and encoded amino acid sequence (SEQ ID No: 2) of the mutated truncated RSV F gene is shown in Figure 2.

20 The alphavirus preferably is a Semliki Forest virus and the first DNA sequence is the Semliki Forest viral sequence contained in plasmid pSFV1. The promoter used preferably is the SP6 promoter.

25 The vector may contain a unique restriction site permitting linearization of the vector without clearing the second nucleotide sequence and maintaining the first and second nucleotides sequences under transcriptional control of the promoter. The unique restriction site preferably is a SpeI site, particularly that derived 30 from pSFV1. The linearized form of the vector forms an embodiment herein.

The vector may be a plasmid vector, preferably one having the identifying characteristics of plasmid pMP37 (ATCC 97905) as shown in Figure 1C and, more preferably, 35 is the plasmid pMP37.

The mutant DNA sequence encoding an RSV F protein or a fragment thereof capable of inducing antibodies that specifically react with RSV F protein and lacking the SpeI restriction site present in the native DNA 5 sequence, constitutes another aspect of the present invention. Such mutant DNA sequence lacking the SpeI site of the native sequence preferably is that shown in Figure 2 (SEQ ID No: 1) or one which encodes the amino acid sequence shown in Figure 2 (SEQ ID No: 2).

10 The novel vector provided herein may be linearized by cleavage at the unique restriction site and transcribed to an RNA transcript. In accordance with a further aspect of the invention, there is provided an RNA transcript of a vector as provided herein produced 15 by linearization and transcription.

20 The RNA transcripts provided herein may be provided in the form of an immunogenic composition for *in vivo* administration to a host for the generation in the host of antibodies to paramyxovirus protein, such immunogenic compositions comprising, as the active component thereof, an RNA transcript as provided herein. Such immunogenic compositions, which are provided in accordance with another aspect of the invention, may be formulated with any suitable pharmaceutically-acceptable 25 carrier for the *in vivo* administration and may produce a protective immune response.

30 In a yet further aspect of the present invention, there is provided a method of immunizing a host against disease caused by infection with paramyxovirus, which comprises administering to the host an effective amount of an RNA transcript as provided herein.

35 The present invention also includes a novel method of using a gene encoding an RSV F protein or a fragment of an RSV F protein capable of generating antibodies which specifically react with RSV F protein to protect a host against disease caused by infection with

respiratory syncytial virus, which comprises isolating said gene; operatively linking said gene to a DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of
5 complete alphavirus RNA genome replication regions in a region of said DNA sequence which is non-essential for replication to form a plasmid vector wherein said gene and DNA sequence are under transcriptional control of a promoter; linearizing the plasmid vector while
10 maintaining said gene and DNA sequence under said transcriptional control of the promoter; forming an RNA transcript of said linearized vector; and introducing said RNA transcript to said host.

Linearizing the plasmid vector is effected by
15 clearing the plasmid vector at a unique restriction site therein at a location which permits the maintenance of the gene and the DNA sequence under the transcriptional control of the promoter. The unique restriction site may be an SpeI site, such as derived
20 from plasmid pSFV1.

The plasmid vector employed preferably is plasmid pMP37 and the linearizing step is effected by cleavage at the SpeI site of plasmid pMP37 (see Figure 1C).

In addition, the present invention includes a
25 method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises isolating a first DNA sequence encoding an RSV F protein from which the transmembrane anchor and cytoplasmic tail are
30 absent and lacking any SpeI restriction site; operatively linking said first DNA sequence to a second DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complete alphavirus genome replication regions in a region of
35 said second DNA sequence which is non-essential for replication to form a plasmid vector wherein said first

and second DNA sequences are under transcriptional control of a promoter; linearizing the plasmid vector while maintaining said first and second DNA sequences under said transcriptional control of the promoter; 5 forming a RNA transcript of said linearized vector; and formulating said RNA transcript as a vaccine for *in vivo* administration.

Linearizing the plasmid vector is effected by clearing the plasmid vector at a unique restriction 10 site therein at a location which permits the maintenance of the gene and the DNA sequence under the transcriptional control of the promoter. The unique restriction site may be an SpeI site, such as derived from plasmid pSFV1.

15 The plasmid vector employed preferably is plasmid pMP37 and the linearizing step is effected by cleavage at the SpeI site of plasmid pMP37.

20 Advantages of the present invention include the provision of RNA transcripts which are useful in generating an immune response by *in vivo* administration.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further understood 25 from the following description with reference to the drawings, in which:

Figures 1A, 1B and 1C show a scheme for construction of plasmid pMP37 used to generate the RSV-F RNA;

Figure 2 shows the nucleotide sequence (SEQ ID No: 30 1) and deduced amino acid sequence (SEQ ID No: 2) of a truncated RSV F gene lacking the transmembrane anchor and cytoplasmic tail and mutated at nucleotide 194 to eliminate the SpeI restriction site present in the unmutated gene;

35 Figure 3, comprising panels A, B and C, shows the anti-RSV F titres in sera from mice taken 4 weeks after

primary immunization and 2 weeks after boosting with the RSV F RNA. Panels A, B, and C show total IgG response, IgG1 response and IgG2a response respectively; and

5 Figure 4 shows the RSV-specific neutralizing antibody titres expressed as plaque reduction titres for various RSV preparations.

GENERAL DESCRIPTION OF INVENTION

As described above, the present invention, in general, relates to protection of hosts against disease caused by infection by paramyxovirus by RNA immunization using RNA transcripts formed from DNA vectors by linearization and transcription of the linearized vector. In particular, the invention is concerned with protection of hosts against disease caused by infection by respiratory syncytial virus (RSV), although not specifically limited thereto. The description which follows refers specifically to employing DNA sequences and RNA transcripts thereof encoding RSV F protein and fragments thereof which generate antibodies which specifically react with RSV F protein.

In this application, the term "RSV F protein" is used to define a full-length RSV F protein, including proteins having variations in their amino acid sequences including those naturally occurring in various strain of RSV and those introduced by PCR amplification of the encoding gene while retaining the immunogenic properties, a secreted form of the RSV F protein lacking a transmembrane anchor and cytoplasmic tail, as well as fragments of RSV F protein capable of generating antibodies which specifically react with RSV F protein and functional analogs of RSV F protein. In this application, a first protein is a "functional analog" of a second protein if the first protein is immunologically related to and/or has the same function as the second protein. The functional analog may be,

for example, a fragment of the protein or a substitution, addition or deletion mutant thereof.

A vector is constructed to contain a first DNA sequence which is complementary to at least part of an alphavirus RNA genome, specifically Semliki Forest virus, and having the complement of complete alphavirus RNA genome replication regions. A second DNA sequence encoding the RSV F protein is inserted into a region of the first DNA sequence which is non-essential for replication. The first and second DNA sequences are under transcriptional control of a promoter. The resulting vector is linearized and an RNA transcript formed from the linearized vector.

The RNA transcripts provided herein, when administered to an animal, including a human, replicate rapidly and effect *in vivo* RSV F protein expression, as demonstrated by an RVF-protein specific antibody response in the animal to which it is administered. Such antibodies may be employed, if desired, in the detection of RSV protein in a sample.

As may be seen from the results detailed in the Examples below, the RNA transcripts provided a high anti-F IgG antibody titre with a IgG1/IgG2a ratio closely following the ratio obtained from immunization with live virus. Immunization with the RNA transcripts protected the animals against live RSV challenge.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of RSV infections. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the RSV F gene and vectors as disclosed herein. The vaccine elicits an

immune response in a subject which includes the production of anti-RSVF antibodies. Immunogenic compositions, including vaccines, containing the RNA transcripts may be prepared as injectables, in 5 physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The RNA transcripts associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 10 93/24640, ref. 38) or the RNA may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting 15 in liposome/nucleic acid complexes that capture up to loot of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 20 describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be 25 used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has 30 been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

35 U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet

hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactide-co-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, 5 polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive 10 microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain an absorption enhancer.

15 The RNA transcripts may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and 20 vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, 25 intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an 30 immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral 35 formulations may be desirable. For suppositories, binders and carriers may include, for example,

polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

5 The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject
10 to be treated, including, for example, the capacity of the individual's immune system to synthesize the RSV F protein and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered
15 depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1 µg to about 10 mg of the RSV F RNA. Suitable regimes for initial administration and booster doses are also
20 variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent
25 vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from
30 combinations of various pathogens.

35 Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen

locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen 5 depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have been 10 identified that enhance the immune response to antigens. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only 15 aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines.

A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed 20 to membrane protein antigens to produce immune stimulating complexes (ISCOMS), plutonic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as 25 well as monophoryl lipid A, QS 21 and polyphosphazene.

In particular embodiments of the present invention, the RNA transcript comprising a first nucleotide sequence encoding an F protein of RSV may be delivered 30 in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The RNA transcript may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 39) disclosed that introduction of gold microprojectiles 35 coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH

antibodies in the mice, while Furth et al. (ref. 40) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

Biological Deposits

5 Certain vectors that contain the gene encoding RSV F protein and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., pursuant to the Budapest Treaty and prior
10 to the filing of this application.

Samples of the deposited plasmids will become available to the public upon grant of a patent based upon this United States patent application and all restrictions on access to the deposits will be removed
15 at that time. Non-viable deposits will be replaced in the event ATCC is unable to dispense the same. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration
20 of the invention. Any equivalent or similar plasmids that encode similar or equivalent antigens as described in this application are within the scope of this invention.

Deposit Summary

	<u>Plasmid</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
25	pMP37	97905	Feb. 27, 1997

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can
30 be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as
35 circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms

are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry and immunology used but not explicitly 5 described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

10 This Example describes the construction of a Semliki Forest virus (SFV) expression vector containing a truncated version of the RSV F gene.

A truncated version of the RSV F gene was inserted 15 into the SFV expression vector pSFV1 (Gibco BRL, Gaithersburg, MD, USA) according to the steps outlined in Figure 1. The RSV F gene was originally cloned from 20 a subtype A RSV clinical isolate into plasmid pRSV F, as fully described in copending United States Patent Application No. 08/001,554 filed January 6, 1993, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference, (ref. 41 and WO 93/14207). A fragment of the RSV F gene was excised 25 from plasmid RSV F by digesting the plasmid with BspHI and EcoRI. The restriction enzyme BspHI cuts within the RSV F gene coding region, removing 48 amino acids from the C-terminus of the F protein. These amino acids make up most of the transmembrane domain and the entire 30 cytoplasmic tail. The resulting 1.6 Kb truncated RSV F gene fragment was cloned into the EcoRI-BamHI sites of the Bluescript-based mammalian cell expression vector pMCR20 (Stratagene, La Jolla, CA) in a 3-way ligation with a linker, based upon the following sequence:

5' CATGACTTGATAATGAG 3' (SEQ ID No: 3)

3' TGAACATTACTCCTAG 5' (SEQ ID No: 4)

35 to generate plasmid pES13A, as described in the aforementioned USAN 08/001,554 (WO 93/14207). This linker adds a non-template encoded threonine to the

truncated RSV F protein C-terminus and inserts three successive stop codons at the end of the truncated gene.

The 1.6 Kb truncated RSV F gene fragment was then excised from plasmid pES13A by digesting with EcoRI and 5 BamHI. In another 3-way ligation, the 1.6 Kb EcoRI-BamHI RSV F gene fragment was cloned into the BamHI site of the SFV expression vector pSFV1 with another linker, based upon the following sequence:

10 5' GATCCGCGCGCGCG 3' (SEQ ID No: 5)
 3' GCGCGCGCGCTTAA 5' (SEQ ID No: 6)

to generate plasmid pMP35. This plasmid contained two copies of the 1.6 Kb BamHI RSV F gene fragment. At this time, it was discovered that there was an SpeI site located in the RSV F gene fragment 193 bp from the 15 upstream BamHI site. It is necessary to linearize a pSFV1 based plasmid with SpeI prior to its use in the in vitro transcription reaction described below. Therefore, the SpeI site in the RSV F gene needed to be removed and this was effected in the following manner.

20 The 1.6 Kb truncated RSV F gene fragment was excised from plasmid pMP35 by digesting with BamHI and ligated into the BamHI site of pUC19 to generate the plasmid pMP36. The Transformer™ site-directed mutagenesis kit (Clonetech, Palo Alto, CA, USA) and a 25 primer, 5'- TGGTTGGTATACCAAGTGTATAACT (SEQ ID No: 7) were used, according to the manufacturer's instructions, to change nucleotide 194 from a T to a C. This change eliminates the SpeI site in the RSV F gene without affecting the amino acid sequence of the encoded 30 RSV F protein. The sequence of plasmid pMP36A, which contains the altered RSV F gene, was determined by DNA sequence analysis. The 1.6 Kb truncated RSV F gene fragment was excised from plasmid pMP36A by digesting with BamHI and ligated into the BamHI site of pSFV1 to 35 generate plasmid pMP37 (ATCC 97905). Proper orientation of the truncated RSV F gene was confirmed by restriction

mapping and DNA sequence analysis. Figure 2 shows the nucleotide sequence (SEQ ID No: 1) of the truncated RSV F gene BamHI fragment with the SpeI site eliminated and the amino acid sequence (SEQ ID No: 2) of the secreted
5 RSV F protein encoded thereby.

Plasmid DNA was purified using plasmid DNA mide kits from Qiagen (Chatsworth, CA, USA), according to the manufacturer's instructions.

Example 2

10 This Example describes the preparation of SpeI linearized pMP37 required for the generation of SFV-RSVF RNA in *in vitro* transcription reactions and the preparation of SFV-RSVF RNA.

15 20 µg of plasmid pMP37, prepared as described in Example 1, was cut with SpeI in a 100 µL reaction containing 20 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 50 mM KC1 and 30 units of SpeI (Gibco BRL, Gaithersburg, MD, USA).

20 SFV-RNA was generated from the linearized plasmid in a 300 µL *in vitro* transcription reaction using of the following materials :

25 40 mM Tris-HCl (pH 7.9)
 6 mM MgCl₂
 2 mM spermidine-(HCl)₃
 1 mM DTT (dithiothreitol)
 1 mM ATP (adenosine triphosphate)
 1 mM GTP (guanosine triphosphate)
 1 mM CTP (cytidine triphosphate)
 1 mM UTP (uridine triphosphate)
30 1 mM m⁷G(5')ppp(5')G RNA cap analog (New England Biolabs, Mississauga, Ont., Canada)
 360 units of RNasin® enzyme inhibitor (Promega, Madison, WI, USA)
 270 units of SP6 RNA polymerase (Gibco BRL, Gaithersburg, MD, USA)

The reaction was incubated at 37°C for 50 minutes. The SFV-RSVF RNA so produced was purified from the salt, enzymes, unincorporated NTP's and cap analog by passing the reaction mix through CHROMA SPIN™-200 DEPC-H₂O columns (Clonetech, Palo Alto, CA, USA) (75 µL/column) according to the manufacturer's instructions. The purified RNA then was ethanol precipitated and resuspended in DEPC-treated H₂O to a final concentration of 1 µg/µL. The purified RNA was mixed with an equal volume of 2X PBS just prior to immunization.

Example 3

This Example describes the immunization of mice with SFV-RSVF RNA and the immunogenicity results obtained.

It has previously been shown that mice are susceptible to infection with RSV (ref. 42) and are a relevant animal model. The mice were immunized with the SFV-RSVF RNA prepared as described in Example 2, by the intramuscular (i.m.) route. The anterior tibialis muscles of five BALB/c mice (female 6 to 8 week old) (Jackson Lab., Bar Harbour, ME, USA) were bilaterally injected with 2 x 25 µg (0.5 µg/µL) of the PBS-directed SFV-RSVF RNA. Five days prior to RNA immunization, the muscles were treated with 2 x 50 µL of cardiotoxin (10 µM in PBS) (Latoxan, France). Treatment of muscles with cardiotoxin has previously been shown to enhance the uptake of DNA and enhance the immune response (ref. 43). These mice were boosted in an identical manner 4 weeks later (Table 1 below). The control groups were immunized with (1) SFV RNA expressing β-galactosidase (SFV-LacZ RNA), (2) SFV-RSVF RNA as prepared herein; (3) live RSV, (4) PBS with alum and RSV subunit preparation with alum. These mice were also boosted in an identical manner 4 weeks later (Table 1). The RSV subunit preparation, comprising RSV F, G and M proteins is described in

copending United States Patent Application No. 08/679,060 filed July 12, 1996, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference (WO 98/02457).

5 Two weeks after the second immunization, mice were challenged intranasally with 10^6 plaque forming units (pfu) of the A2 strain of RSV (BG-4A). Animals were sacrificed 4 days later. Lungs were aseptically removed, weighed, and homogenized in 2 mL of complete culture medium. The virus titre in lung homogenates was determined in duplicate using Vero cells, as previously described (ref. 44).

10 Sera was obtained from the mice at 4 and 6 weeks. Anti-RSV F antibody titres (IgG, IgG1 and IgG2a) in these sera were determined by enzyme-linked immunosorbent assay (ELISA), as described in Example 4. The RSV-specific plaque reduction titres of these sera were determined as previously described (ref. 44).

15 The anti-RSV F antibody responses in the sera of BALB/c mice that were immunized as outlined in Table 1 are summarized in Figure 3. The animals immunized with SFV-RSVF RNA, live RSV, or RSV subunit preparation + alum all had high total anti-RSV F IgG antibody titres in their serum at both 4 and 6 weeks (Figure 3, panel A). However, the IgG1/IgG2a ratios differed markedly, as seen from Figure 3, panels B and C. The sera from animals that were immunized with live RSV had an anti-F IgG1/IgG2a ratio of approximately 0.69 after 6 weeks. This value is in contrast to the anti-RSV F IgG1/IgG2a 20 ratio obtained in mice after 6 weeks that were primed and boosted with the alum-adjuvanted RSV subunit preparation. In this case, the anti-RSV IgG1/IgG2a ratio was approximately 4.3. The anti-RSV F IgG1/IgG2a 25 ratios obtained in mice immunized with SFV-RSVF RNA after 6 weeks were 0.79. These results suggest that immunization of mice with the SFV-RSVF RNA results in 30 35

more of a Th-1 type response similar to that obtained with live RS virus rather than the Th-2 type response seen with the alum-adjuvanted RSV subunit preparation.

As shown in Figure 4, the sera of mice that were primed and boosted with the various RSV preparations as outlined in Table 1, all had significant levels of RSV-specific neutralizing antibodies (groups 2, 3 and 5). In contrast to the placebo control animals (groups 1 and 4), the lower respiratory tract of mice that were immunized with SFV-RSVF RNA, live RSV, or the alum-adjuvanted RSV subunit preparation, were completely protected against live RS virus challenge, as seen in Table 2.

Immunization of mice with the SFV-RSVF RNA protected mice against live RSV challenge. The protective ability of this SFV replicon was comparable to that induced by inoculation with live RSV or alum--adjuvanted RSV subunits. The type of immune response generated appeared to be more of a Th-1 like response similar to that elicited by live RSV.

Example 4

This Example describes the determination of anti-RSV F antibody titres.

Nunc-MaxiSorp plate wells were coated overnight at room temperature with 2.5 ng of immunoaffinity-purified RSV F protein diluted in 0.05M carbonate-bicarbonate buffer, pH 9.6. Wells were blocked for non-specific binding by adding 0.1% BSA in PBS for 30 min. at room temperature, followed by two washes in a washing buffer of 0.1% BSA in PBS + 0.1% Tween 20. Serial two or four-fold dilutions of mouse serum was added to the wells. After a one hour incubation at room temperature, plates were washed five times with washing buffer, and horseradish peroxidase (HRP) labeled conjugate was added at the appropriate optimal dilution in washing buffer. The total IgG assay used F(ab')₂ goat antimouse IgG (H+L

specific)-HRP from Jackson Immuno Research Laboratory Inc. (Baltimore MD, USA). Sheep anti-mouse IgG1-HRP from Serotec (Toronto, Ontario, Canada) was used in the IgG1 assay and goat anti-mouse IgG2a from Caltag Laboratories (San Francisco, CA, USA) was used in the IgG2a assay. Following one hour incubation at room temperature, the plates were washed five times with washing buffer, and hydrogen peroxide (substrate) in the presence of tetramethylbenzidine was added. The reaction was stopped by adding 2 M sulfuric acid. The colour was read in a Multiscan Titertek plate reader at an optical density (OD) of 450 nm. The titre was taken as the reciprocal of the last dilution at which the OD was approximately double. This OD must be greater than the negative control of the assay at the starting dilution. The pre-immune serum of each animal was used as the negative control.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, there are provided novel vectors containing DNA sequences encoding a paramyxovirus protein, particularly a RSV F protein, which can be linearized and transcribed to RNA for *in vivo* administration to generate a protective immune response to disease caused by infection by paramyxovirus, particularly respiratory syncytial virus. Modifications are possible within the scope of this invention.

Table 1. Immunization protocol

GROUP	PRIME	ROUTE OF INOCULATION	BOOST	ROUTE OF INOCULATION
1	SFV-LacZ RNA ¹	Intramuscular	SFV-LacZ RNA ¹	Intramuscular
2	SFV-RSVF RNA ¹	Intramuscular	SFV-RSVF RNA ¹	Intramuscular
3	Live RSV ²	Intranasal	Live RSV ²	Intranasal
4	PBS + alum	Intramuscular	PBS + alum	Intramuscular
5	RSV subunits + alum ³	Intramuscular	RSV subunits + alum ³	Intramuscular

Mice were inoculated with:

1. 25 µg of RNA was injected into each hind leg muscle in 50 µL of PBS
2. 2.5 X 10⁵ pfu of mouse-adapted A2 virus
3. 1 µg of RSV subunit vaccine adsorbed to alum (1.5 mg/dose)

Table 2

GROUP	Antigen Formulation		Mean Virus Lung Titre (log ₁₀ /g ± s.d.)	% Protection
	Prime	Boost		
1	SFV-LacZ RNA	SFV-LacZ RNA	4.18 ± 0.06	0
2	SFV-RSVF RNA	SFV-RSVF RNA	≤ 1.83 ± 0	100
3	Live RSV	Live RSV	≤ 1.83 ± 0	100
4	PBS + alum	PBS + alum	4.17 ± 0.17	0
5	RSV subunits + alum	RSV subunits + alum	≤ 1.83 ± 0	100

REFERENCES

1. McIntosh K. and Chanock R.M. in Fields B.N. and Knipe D.M. (eds). *Virology*. Raven Press, New York, 1990, pp.1045-1072.
2. Murphy B.R., Hall S.L., Kulkarni A.B., Crowe J.E.; Collins P.L., Connors M., Karzon R.A. and Chanock R.M., *Virus Res* 32, 13-36, 1994.
3. Osterweil D. and Norman D., *Am Geriat Soc* 36, 659-662, 1990.
4. Agius G., Dindinand G., Biggar R.J., Peyre R., Vaillant V., Ranger S., Poupet J.Y., Cisse M.F. and Casters M., *J Med Virol* 30, 117-127, 1990.
5. Katz S.L. in *New vaccine development establishing priorities* Vol 1. National Academic Press, Washington, 1985, pp. 3974 09.
6. Wertz G.W. and Sullender W.M., *Biotechnology* 20, 151-176, 1992 .
7. Fulginiti V.A., Eller J.J., Sieber O.F., Joyner J.W., Minamitani M. and Meiklejohn G., *Am J Epidemiol* 89, 449-463, 1969.
- B. Chin J., Magoffin R.L., Shearer I.A., Schieble J.H. and Lennette E.H., *Am J Epidemiol* 89, 449-463, 1969.
9. Belshe R.B., Van Voris L.P. and Mufson M.A., *J Infect Dis* 145, 311-319, 1982.
10. Kim R.M., Arrobio J.O., Pyles G., Brandt C.D., Camargo E., Chanock R.M. and Parrott R.H., *Pediatrics* 48, 745-755, 1971.
11. Gruber C. and Levine S., *J Gen Virol* 64, 825-832, 1983.
12. Olmstead R.A., Elango N. and Prince G.A., *Proc Natl Acad Sci USA* 83, 7462-7466, 1991.
13. Parrington M., Cockle S., Wyde P., Du R.-P., Snell E., Yan W.-Y., Wang Q., Gisonni L., Sanhueza S., Ewaszyn M. and Klein M., *Virus Genes* 14, 65-74, 1997
14. Fulginiti, V.A., Eller, J.J., Sieber, O.F., Joyner, J.W., Minamitani, M. and Meiklejohn, G. (1969) *Am. J. Epidemiol.* 89 (4), 435-448.

15. Chin, J., Magoffin, R.L., Shearer, L.A., Schieble, J.H. and Lennette, E.H. (1969) Am. J. Epidemiol. 89 (4), 449-463.
16. Jensen, K.E., Peeler, B.E. and Dulworth, W.G. (1962) J. Immunol. 89, 216-226.
17. Murphy, B.R., Prince, G.A., Collins, P.L., Van Wyke Coelingh, K., Olmsted, R.A., Spriggs, M.K., Parrott, R.H., Kim, H.-Y., Brandt, C.D. and Chanock, R.M. (1988) Vir. Res. 11, 1-15.
18. Hall, S.L., Sarris, C.M., Tierney, E.L., London, W.T., and Murphy, B.R. (1993) J. Infect. Dis. 167, 958-962.
19. Belshe, R.B., Karron, R.A., Newman, F.K., Anderson, E.L., Nugent, S.L., Steinhoff, M., Clements, M.L., Wilson, M.H., Hall, S.L., Tierney, E.L. and Murphy, B.R. (1992) J. Clin. Microbiol. 30 (8), 2064-2070.
20. Hall, S.L., Stokes, A., Tierney, E.L., London, W.T., Belshe, R.B., Newman, F.C. and Murphy, B.R. (1992) Vir. Res. 22, 173-184.
21. Van Wyke Coelingh, K.L., Winter, C.C., Tierney, E.L., London, W.T. and Murphy, B.R. (1988) J. Infect. Dis. 157 (4), 655-662.
22. Ray, R., Novak, M., Duncan, J.D., Matsuoka, Y. and Compans, R.W. (1993) J. Infect. Dis. 167, 752-755.
23. Ray, R., Brown, V.E. and Compans, R.W. (1985) J. Infect. Dis. 152 (6), 1219-1230.
24. Ray, R. and Compans, R.W. (1987) J. Gen. Virol. 68, 409-418.
25. Ray, R., Glaze, B.J., Moldoveanu, Z. and Compans, R.W. (1988) J. Infect. Dis. 157 (4), 648-654.
26. Ray, R., Matsuoka, Y., Burnett, T.L., Glaze, B.J. and Compans, R.W. (1990) J. Infect. Dis. 162, 746-749.
27. Ray, R., Glaze, B.J. and Compans, R.W. (1988) J. Virol. 62 (3), 783-787.
28. Ewasyshyn, M., Caplan, B., Bonneau A.-M., Scollard, N., Graham, S., Usman, S. and Klein, M. (1992) Vaccine 10 (6), 412-420.

29. Ambrose, M.W., Wyde, P.R., Ewasyshyn, M., Bonneau, A.-M., Caplan, B., Meyer, H.L. and Klein, M. (1991) Vaccine 9, 505-511.
30. Kasel, J.A., Frank, A.L., Keitel, W.H., Taber, L.H., Glezen W.P. J. Virol. 1984; 52:828-32.
31. Lehman, D.J., Roof, L.L., Brideau, R.J., Aeed, P.A., Thomsen, D.R., Elhammer, A.P., Wathen, M.W. and Homa, F.L. (1993) J. Gen. Virol. 74, 459-469.
32. Brideau, R.J., Oien, N.L., Lehman, D.J., Homa, F.L. and Wathen, M.W. (1993) J. Gen. Virol. 74, 471-477.
33. Ebata, S.N., Prevec, L., Graham, F.L. and Dimock, K. (1992) Vir. Res. 24, 21-33.
34. Hall, S.L., Murphy, B.R. and Van Wyke Coelingh, K.L. (1991) Vaccine 9, 659-667.
35. Strauss E.G. and Strauss J.H., in Schlesinger S.S. and Schlesinger M.i. (eds). The Togaviridae and Flaviviridae. Plenum Press, New York, 1986, pp.35-90.
36. Liljestrom P. and Garoff H., Biotechnology 9, 1356-1361, 1991.
37. Zhou X., Berglund P., Rhodes G., Parker S.E., Jondal M. and Liljestrom P., Vaccine 12, 1510-1514, 1994.
38. Dalemans W., Delers A., Delmelle C., Denamur F., Meykens R., Thiriart C., Veenstra S., Francotte M., Bruck C. and Cohen J., Annals New York Academy of Sciences , 255-256, 1996.
39. Tang et al, Nature 1992, 356: 152-154.
40. Futh et al, Analytical Biochemistry, 1992, 205: 365-368.
41. Du, R.P. et al., Biotechnology 12, 813-818, 1994
42. Graham B.S., Perkins M.D., Wright P.F. and Karzon D.T., J. Mod. Virol. 26, 153-162, 1988.
43. Davis et al. Vaccine 12, 1503-1509, 1994.
44. Prince, G.A., et al. Am. J. Pathol. 93, 771-790, 1978.

CLAIMS

What we claim is:

1. A vector, comprising:

a first DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of complete alphavirus RNA genome replication regions,

a second DNA sequence encoding a paramyxovirus protein or a protein fragment that generates antibodies that specifically react with the paramyxovirus protein,

said second DNA sequence being inserted into a region of said first DNA sequence which is non-essential for replication thereof,

said first and second DNA sequences being under transcriptional control of a promoter.

2. The vector of claim 1 wherein said paramyxovirus protein is selected from the group consisting of a parainfluenza virus (PIV) protein and a respiratory syncytial virus (RSV) protein.

3. The vector of claim 2 wherein said paramyxoviridae protein is a PIV protein selected from the group consisting of PIV-1, PIV-2, PIV-3 and PIV-4 protein.

4. The vector of claim 3 wherein the PIV protein is selected from the group consisting of the HN and F glycoproteins of PIV-3.

5. The vector of claim 2 wherein said paramyxoviridae protein is an RSV protein.

6. The vector of claim 5 wherein said RSV protein is selected from the group consisting of the F and G glycoproteins of RSV.

7. The vector of claim 1 wherein said second DNA sequence encodes a full-length RSV F protein.

8. The vector of claim 1 wherein said second DNA sequence encodes a RSV F protein lacking the transmembrane anchor and cytoplasmic tail.

9. The vector of claim 1 wherein said second DNA sequence encodes a RSV F protein and lacks a Spe I restriction site.
10. The vector of claim 1 wherein said second DNA sequence encodes a RSV F protein and lacks a Spe I restriction site and lacks a transmembrane anchor and cytoplasmic tail encoding region.
11. The vector of claim 10 wherein nucleotide 194 (T) of the RSV F gene is mutated to a C to eliminate the Spe I site in the RSV F gene.
12. The vector of claim 1 wherein said alphavirus is a Semliki Forest virus.
13. The vector of claim 12 wherein said first DNA sequence is the Semliki Forest virus sequence contained in plasmid pSFV1.
14. The vector of claim 12 wherein said promoter is the SP6 promoter.
15. The vector of claim 1 which is a plasmid vector having a unique restriction site permitting linearization of the vector without clearing the second DNA sequence.
16. The vector of claim 15 wherein the unique restriction site is SpeI site.
17. The vector of claim 16 wherein the SpeI site is derived from plasmid pSFV1.
18. The vector of claim 1 is in a linearized form.
19. The vector of claim 1 having the identifying characteristics of plasmid pMP37 (ATCC 97905) shown in Figure 1C.
20. The vector of claim 1 which is plasmid pMP37 (ATCC 97905).
21. A mutant DNA sequence encoding an RSV F protein or a fragment thereof capable of inducing antibodies that specifically react with RSV F protein, from which is about an SpeI restriction site present in the non-mutant sequence.

22. The mutant DNA sequence of claim 21 which lacks a transmembrane anchor and cytoplasmic tail encoding region.
23. The mutant of claim 21 wherein nucleotide 194 (T) of a non-mutant RSV F gene is mutated to a C to eliminate the SpeI site in the non-mutant RSV F gene.
24. A mutant DNA molecule sequence lacking an SpeI site present in the non-mutant sequence and encoding a truncated RSV F protein and having the DNA sequence shown in Figure 2 (SEQ ID No:1) or having a DNA sequence encoding the amino acid sequence shown in Figure 2 (SEQ ID No: 2).
25. An RNA transcript of the vector of claim 1.
26. The RNA transcript of claim 25 which is derived by linearization of a plasmid vector of claim 1 at a unique restriction site provided in the vector and permitting linearization of the vector without clearing the second DNA sequence and transcribing the linearized vector.
27. The RNA transcript of claim 26 wherein said unique restriction site is an SpeI site.
28. The RNA transcript of claim 25 wherein, in said vector, said second DNA sequence encodes a paramyxoviridae protein selected from the group consisting of a parainfluenza virus (PIV) protein and a respiratory syncytial virus (RSV) protein.
29. The RNA transcript of claim 28 wherein, in said vector, said second DNA sequence encodes a paramyxoviridae protein selected from the group consisting of PIV-1, PIV-2, PIV-3 and PIV-4.
30. The RNA transcript of claim 29 wherein, in said vector, said second DNA sequence encodes a paramyxoviridae protein selected from the group consisting of the HN and F glycoproteins of PIV-3.

31. The RNA transcript of claim 28 wherein, in said vector, said second DNA sequence encodes an RSV protein.
32. The RNA transcript of claim 31 wherein, in said vector, RSV protein is selected from the group consisting of the F and G glycoprotein of RSV.
33. The RNA transcript of claim 25 wherein, in said vector, said second DNA sequence encodes a full-length RSV F protein.
34. The RNA transcript of claim 25 wherein, in said vector, said second DNA sequence encodes a RSV F protein lacking the transmembrane anchor and cytoplasmic tail.
35. The RNA transcript of claim 25 wherein, in said vector, said second DNA sequence encodes a RSV F protein and lacks a Spe I restriction site.
36. The RNA transcript of claim 35 wherein, in said vector, said second DNA sequence encodes a RSV F protein and lacks a Spe I restriction site and lacks a transmembrane anchor and cytoplasmic tail encoding region.
37. The RNA transcript of claim 36 wherein, in said vector, nucleotide 194 (T) of the RSV F gene is mutated to a C to eliminate the Spe I site in the native RSV F gene.
38. The RNA transcript of claim 25 wherein, in said vector, said alphavirus is a Semliki Forest virus.
39. The RNA transcript of claim 38 wherein, in said vector, said first DNA sequence is the Semliki Forest virus sequence contained in plasmid pSFV1.
40. The RNA transcript of claim 38 wherein, in said vector, said promoter is the SP6 promoter.
41. The RNA transcript of claim 25, wherein, said vector has the identifying characteristics of plasmid pMP37 (ATCC 97905) shown in Figure 1C.

42. The RNA transcript of claim 25, wherein said vector is plasmid pMP37 (ATCC 97905).
43. An immunogenic composition for *in vivo* administration to a host for the generation in the host of antibodies to paramyxoviridae protein, comprising, as the active component thereof, an RNA transcript as claimed in claim 25.
44. The immunogenic composition of claim 43 for the generation of protective antibodies in the host.
45. A method of immunizing a host against disease caused by infection with paramyxovirus, which comprises administering to said host an effective amount of an RNA transcript as claimed in claim 25.
46. A method of using a gene encoding an RSV F protein, which comprises:
 - isolating said gene,
 - operatively linking said gene to a DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of complete alphavirus RNA genome replication regions in a region of said DNA sequence which is non-essential for replication to form a plasmid vector wherein said gene and DNA sequence are under transcriptional control of a promoter,
 - linearizing the plasmid vector while maintaining said gene and DNA sequence under said transcriptional control of the promoter,
 - forming an RNA transcript of said linearized vector, and
 - introducing said RNA transcript to said host.
47. The method of claim 46 wherein said linearizing of the plasmid vector is effected by clearing the plasmid vector at a unique restriction site therein at a location which permits the maintenance of the gene and DNA sequence under transcriptional control of the promoter.

48. The method of claim 47 wherein the unique restriction site is an SpeI site.

49. The method of claim 46 wherein said vector is plasmid pMP37 (ATCC 97905) and is linearized by cleavage at the SpeI site therein.

50. A method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isолating a first DNA sequence encoding an RSV F protein from which the transmembrane anchor and cytoplasmic tail are absent and lacking any SpeI restriction site,

operatively linking said first DNA sequence to a second DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complete alphavirus genome replication regions in a region of said second DNA sequence which is non-essential for replication to form a plasmid vector wherein said first and second DNA sequences are under transcriptional control of a promoter,

linearizing the plasmid vector while maintaining said first and second DNA sequences under said transcriptional control of the promoter,

forming a RNA transcript of said linearized vector, and

formulating said RNA transcript as a vaccine for *in vivo* administration.

51. The method of claim 50 wherein said linearizing of the plasmid vector is effected by clearing the plasmid vector at a unique restriction site therein at a location which permits the maintenance of the gene and DNA sequence under transcriptional control of the promoter.

52. The method of claim 51 wherein the unique restriction site is an SpeI site.

53. The method of claim 50 wherein said vector is plasmid pMP37 (ATCC 97905) and is linearized by cleavage at the SpeI site.

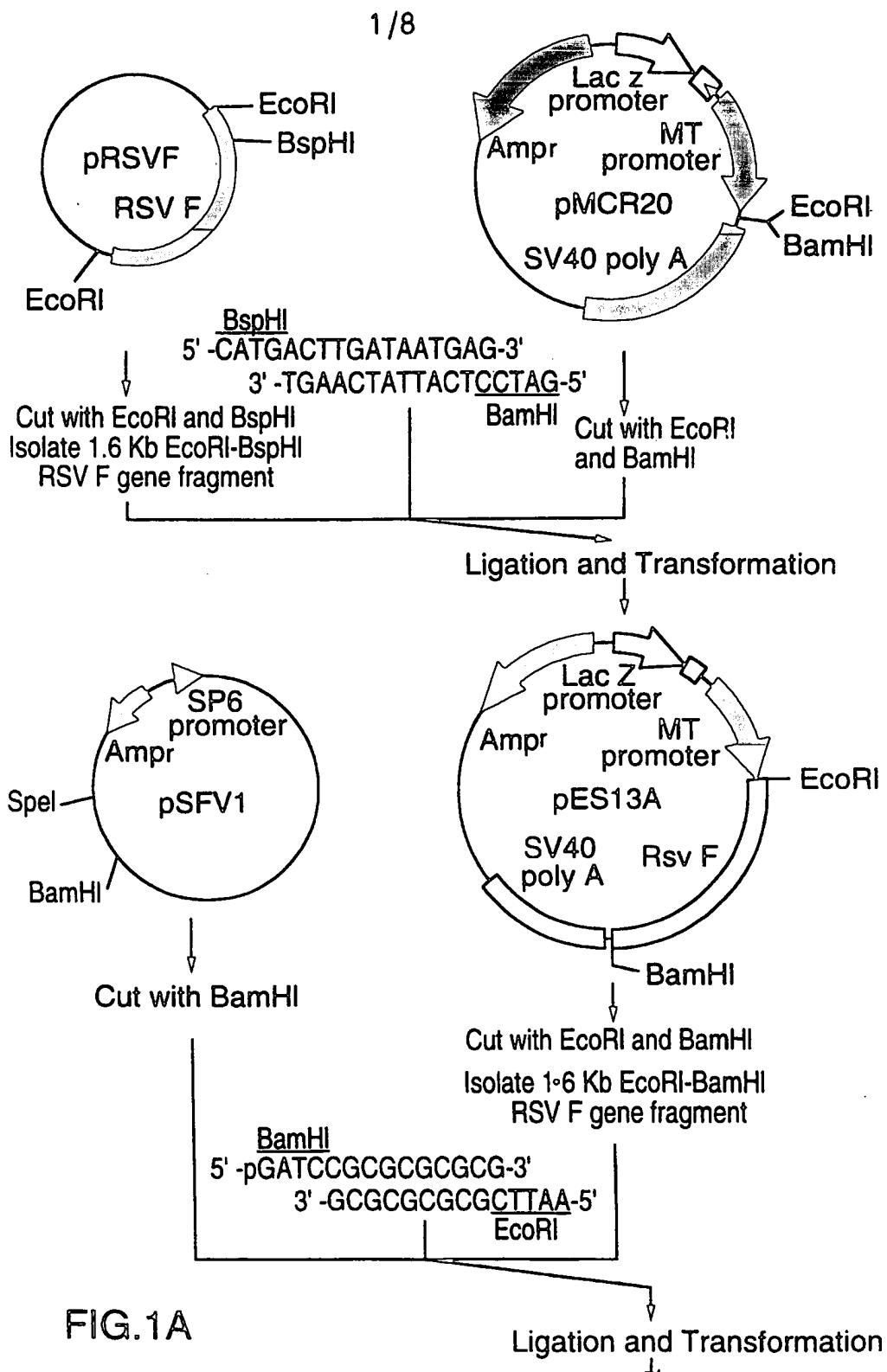
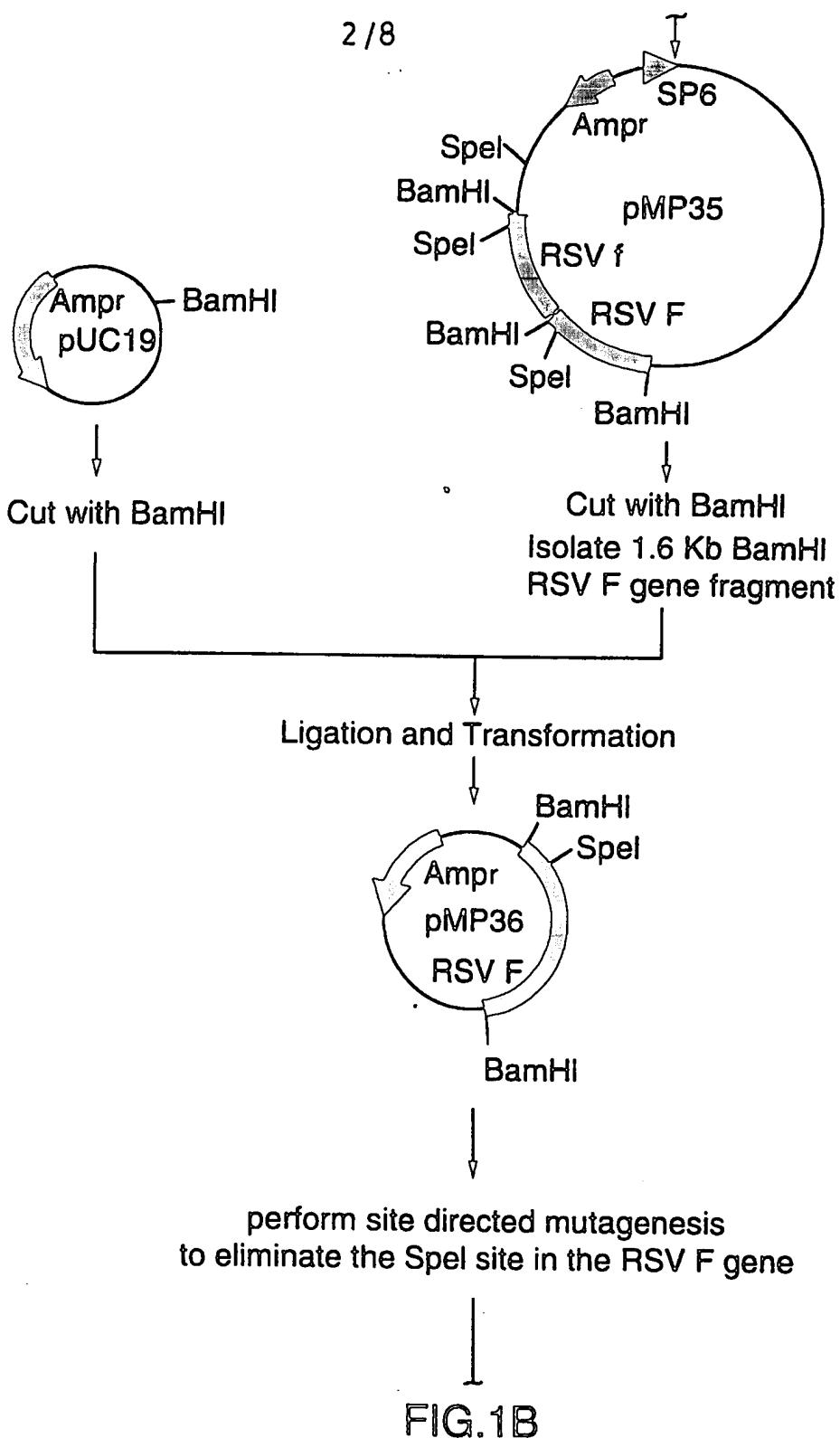


FIG. 1A



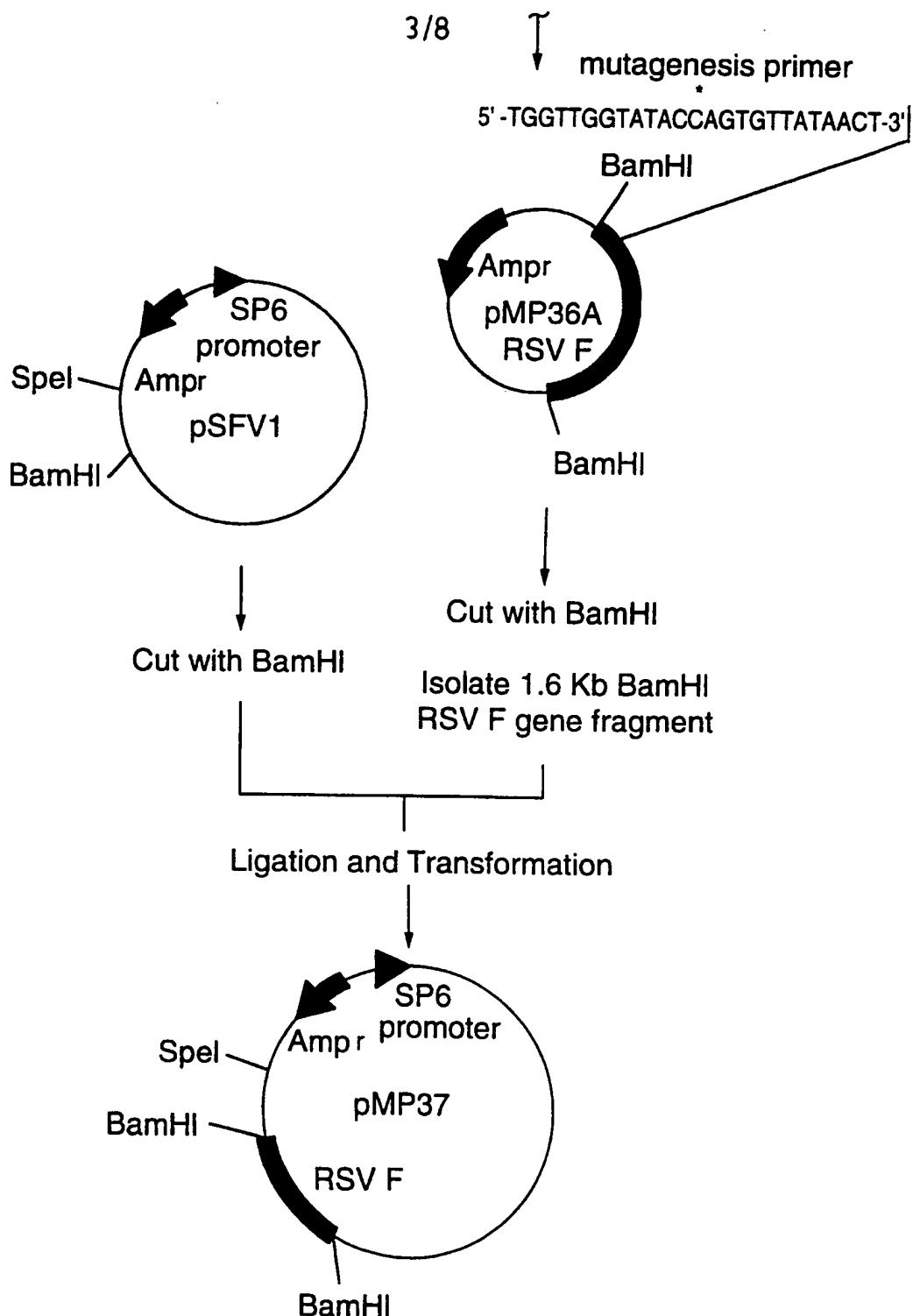


FIG.1C

FIG.2A

0 GATCCGGCGCGAATTGGCACGAGTAACACATTGGAGTTGCTAATCCTCAAAGCAAATG
 0 60
 9
 61 CAATTACCAATCCTCACTGCAGTCACATTTGTTTGCTTCTGGTCAAAACATCACTG 120
 10 29
 121 AAGAATTTTATCAACATGCAGTGCAGTTAGCAAAGGCTATCTTAGTGCTCTGAGAA 180
 30 49
 4 /8
 181 CTGGTTGGTATAACCAGTGTATAACTATAGAATTAAAGTAATATCAAGGAAAATAAGTGT 240
 50 69
 241 ATGGAACAGATGCTAAGGTAAATTGATAAAACAAGAATTAGATAAAATATAAAATGCTG 300
 70 89
 301 TAACAGAATTGCAGTTGCTCATGCAAAGCACACCAACAAACAAATCGAGCCAGAAGAG 360
 90 109
 481 AACTACCAAGGTTTATGAATTACACTCAACAAATGCCAAAAAAACCAATGTAACATTAA 420
 110 129
 421 GCAAGAAAAGGAAAAGAGATTCTTGCTTTTGTAGGTGTTGGATCTGCAATGCCA 480
 130 149
 481 GTGGCGTGTGCTGTATCTAAGGTCTGCACCTAGAAGGGGAAGTGAACAAAGATCAAAGTG 540
 150 169

FIG.2B

541 CTCTACTATCCACAAAGGCTGTAGTCAGCTTATCAAATGGAGTTAGTGCTTAACCA 600
 170
 601 GCAAAGTGTAGACCTCAAAAACATAAGATAAACAAATTGTGTTACCTTATTGTGAACAAGC 660
 190
 661 AAAGCTGCAGCATATCAAATATAGAAACTGTGATAAGAGTTCCAAACAAACAGAC 720
 210
 721 TACTAGAGATTACCAGGGAAATTAGTGTAAATGCAGGGTAACTACACCTGTAAGGCACTT 780 5/8
 230
 781 ACATGTTAACTAATAGTGAATTATTGTCAATTAAATCAATGATAATGCCTATAACAAATGATC 840
 250
 841 AGAAAAAGTTAACATGTCCAACAAATGTTCAAATAGTTAGACAGCAAAGTTACTCTATCATGT 900
 270
 901 CCATAATAAGAGGAAGTCTTAGCATATGTAGTACAATTACCAACTATGGTGTATAG 960
 290
 961 ATACACCCCTGTTGGAAACTACACATCCCCTCTATGTACAACCAAACACAAAAGAAGGGT 1020
 310 D T P C W K L H T S P L C T T N T K E G 329
 1021 CCAACATCTGTTAACAAAGAAACTGACAGGGATGGTACTGTGACAATGCAGGATCAGTAT 1080
 330 S N I C L T D R T G W Y C D N A G S V 349

FIG.2C

1081 CTTTCTCCCCAACAGCTGAAACATGTAAGTTCAAATCGAGTTGGACACAA 1140
 350 S F F P Q A E T C K V Q S N R V F C D T 369

1141 TGAACAGTTAACATTACCAAGTGAATAAATCTCTGCAATGTTGACATATTCAACCCCA 1200
 370 M N S L T L P S E I N L C N V D I F N P 389

1201 AATATGATTGTAAAATTATGACTTCAAAACAGATGTAAGCAGCTCCGTTATCACATCTC 1260
 390 K Y D C K I M T S K T D V S S V I T S 409

1261 TAGGAGCCATTGTTGTCATGGCTATGGCAAAACTAAATGTAACGGCATCCAATAAAAATCGTG 1320
 410 L G A I V S C Y G K T C T A S N K N R 429

1321 GAATCATAAAGACATTTCTAACGGGTGGCATTATGTATCAAAATAAAAGGGATGGACACTG 1380
 430 G I K T F S N G C D Y V S N K G M D T 449

1381 TGTTCTGTAGGTAACACATTATGTAACATTAAAGCAAGAAGGTAAAGTCTCTATGTAA 1440
 450 V S V G N T L Y D P L V F P S D E F D 469

1441 AGGGTGAACCAATAATAATTCTATGACCCATTAGTATTCCCCTCTGATGAATTGGATG 1500
 470 K G E P I I N F Y D P L V F P S D E F D 489

1501 CATCAATAATCTCAAGTCAACGAGAAGATAACCAAGGCCATTAGCATTAATCGTAATCCG 1560
 490 A S I S Q V N E K I N Q S L A F I R K S 509

1561 ATGAATTACATAATGTAATGGTAAATCCACCAATAATCATGACTTGATAAT 1620
 510 D E L L H N V N A G K S T T N I M T * 527

1623 GAG

7/8

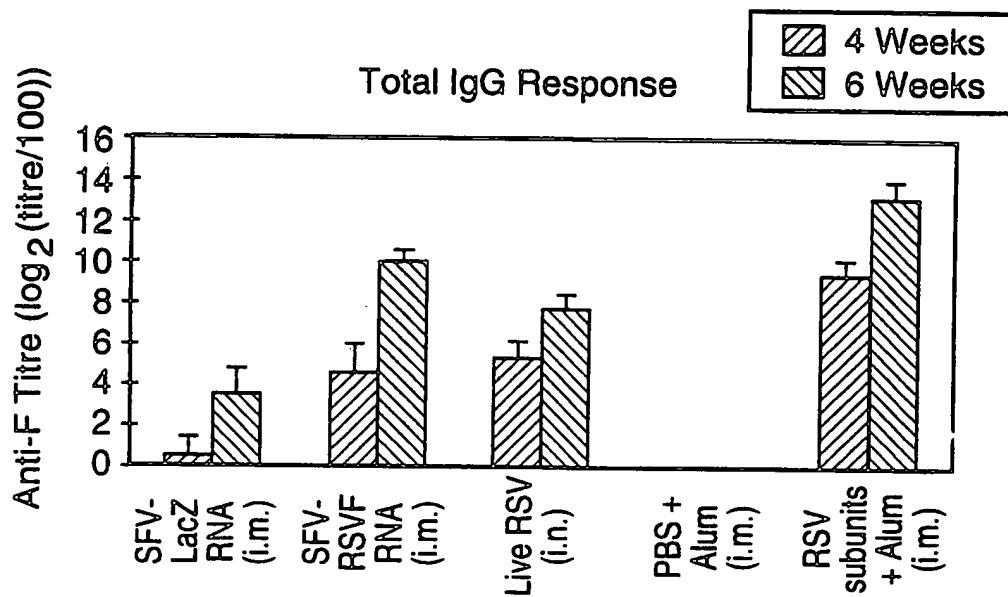


FIG.3A

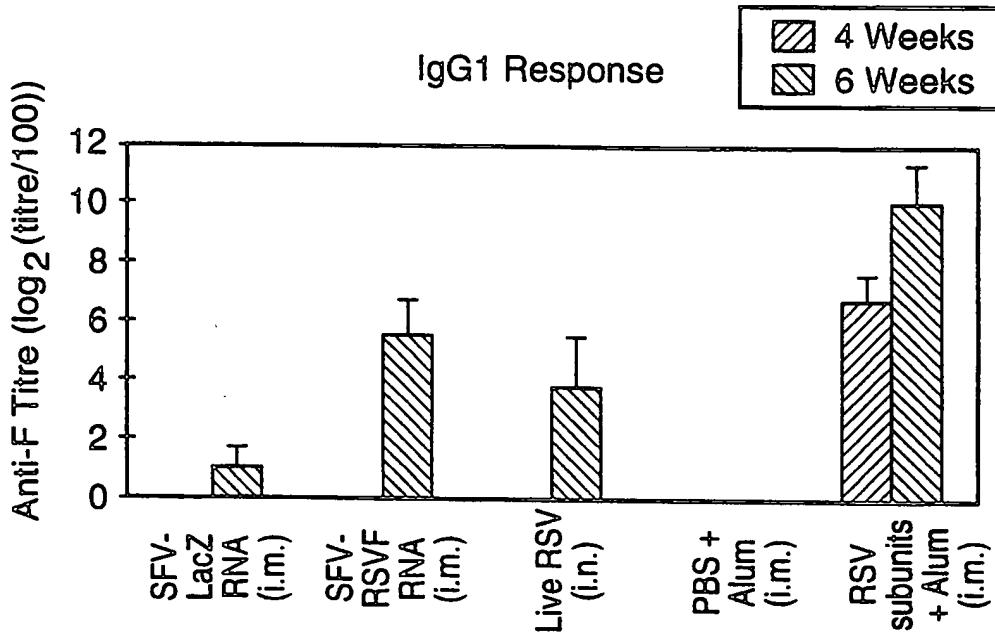


FIG.3B

8 / 8

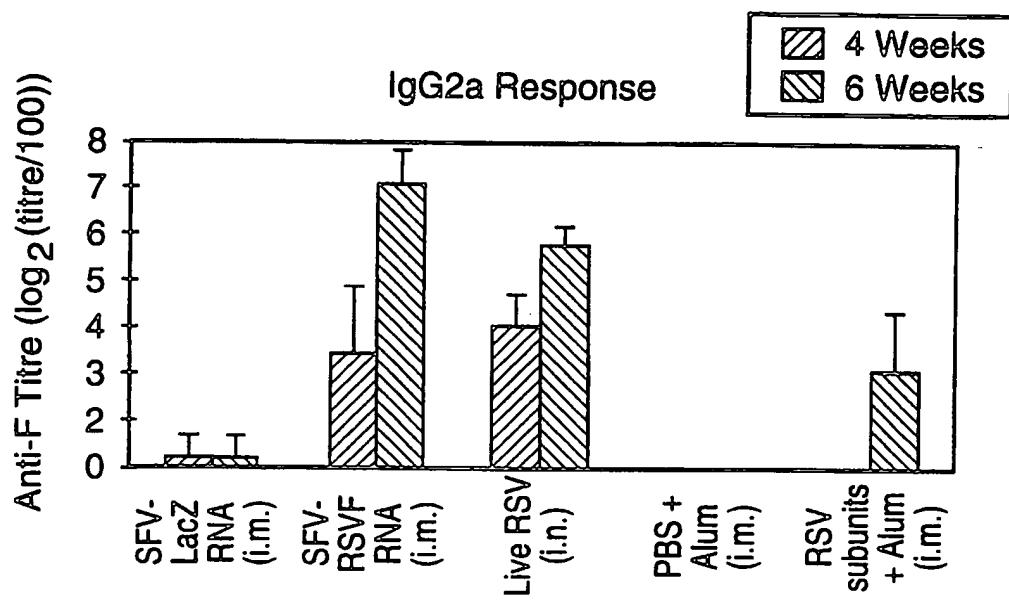


FIG.3C

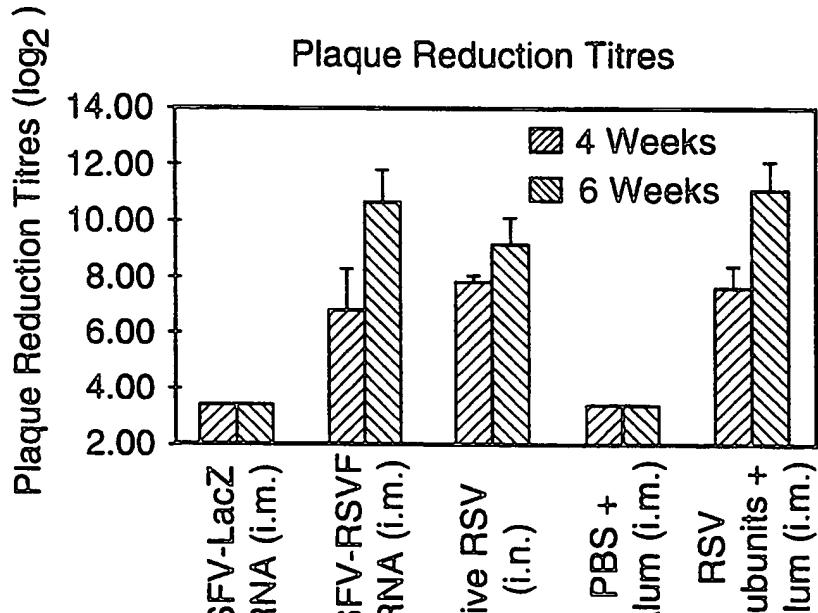


FIG.4

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: CONNAUGHT LABORATORIES LIMITED
 (B) STREET: 1755 Steeles Avenue West
 (C) CITY: North York
 (D) STATE: Ontario
 (E) COUNTRY: Canada
 (F) POSTAL CODE (ZIP): M2R 3T4

(A) NAME: PARRINGTON, Mark
 (B) STREET: 45 Martin Street
 (C) CITY: Bradford
 (D) STATE: Ontario
 (E) COUNTRY: Canada
 (F) POSTAL CODE (ZIP): L3Z 1Z4

(ii) TITLE OF INVENTION: RNA RESPIRATORY SYNCYTIAL VIRUS VACCINES

(iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1623 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GATCCGCGCG CGCGAATTCTG GCACGAGTAA CAATGGAGTT GCTAACCTTC AAAGCAAATG	60
CAATTACCAAC AATCCTCACT GCAGTCACAT TTTGTTTGCT TTCTGGTCAA AACATCACTG	120
AAGAATTTTA TCAATCAACA TGCAGTGCAAG TTAGCAAAGG CTATCTTAGT GCTCTGAGAA	180
CTGGTTGGTA TACCACTATAG AATTAAGTAA TATCAAGGAA AATAAGTGTAA	240
ATGGAACAGA TGCTAAGGTA AAATTGATAA ACAAGAATT AGATAAAATAT AAAAATGCTG	300
TAACAGAATT GCAGTTGCTC ATGCAAAGCA CACCACCAAC AAACAATCGA GCCAGAAGAG	360
AACTACCAAG GTTTATGAAT TATACACTCA ACAATGCCAA AAAAACCAAT GTAACATTAA	420
GCAAGAAAAG GAAAAGAAGA TTTCTTGGTT TTTTGTAGG TGTTGGATCT GCAATGCCA	480
GTGGCGTTGC TGTATCTAAG GTCCTGCACC TAGAAGGGGA AGTGAACAAG ATCAAAAGTG	540
CTCTACTATC CACAAACAAG GCTGTAGTCA GCTTATCAA TGGAGTTAGT GTCTTAACCA	600

GCAAAGTGT	AGACCTAAA	AACTATATAG	ATAAACATT	GTTACCTATT	GTGAACAAGC	660	
AAAGCTGCAG	CATATCAAAT	ATAGAAA	ACTG	TGATAGAGTT	CCAACAAAAG	AACAACAGAC	720
TA	CTAGAGAT	TACCAGGGAA	TTTAGTGT	TA	TCAGGTGT	AACTACACCT	780
ACATGTTAAC	TAATAGTGAA	TTATTGTCAT	TAATCAATGA	TATGCCTATA	ACAAATGATC	840	
AGAAAAAGTT	AATGTCCAC	AATGTTAAA	TAGTTAGACA	GCAAAGTTAC	TCTATCATGT	900	
CCATAATAAA	AGAGGAAGTC	TTAGCATATG	TAGTACAATT	ACCACTATAT	GGTGTATAG	960	
ATACACCC	TTGGAAACTA	CACACATCCC	CTCTATGTAC	ACCAACACA	AAAGAAGGGT	1020	
CCAACATCTG	TTAACAAAGA	ACTGACAGAG	GATGGTACTG	TGACAATGCA	GGATCAGTAT	1080	
CTTTCTTCCC	ACAAGCTGAA	ACATGAAAG	TTCAATCAA	TCGAGTATT	TGTGACACAA	1140	
TGAACAGTTT	AACATTACCA	AGTGAATAA	ATCTCTGCA	TGTTGACATA	TTCAACCCCCA	1200	
AATATGATTG	AAAATTATG	ACTTC	AAA	CAGATGTAA	GAGTCCGTT	ATCACATCTC	1260
TAGGAGCCAT	TGTGT	CATGC	TATGGCAAA	CTAAATGTAC	AGCATCCAAT	AAAATCGTG	1320
GAATCATAAA	GACATTTCT	AAACGGGTGCG	ATTATGTATC	AAATAAAGGG	ATGGACACTG	1380	
TGTCTGTAGG	TAACACATTA	TATTATGTAA	ATAAGCAAGA	AGGTAAAAGT	CTCTATGTAA	1440	
AAGGTGAACC	AATAATAAAT	TTCTATGACC	CATTAGTATT	CCCCTCTGAT	GAATTGATG	1500	
CATCAATATC	TCAAGTCAAC	GAGAAGATTA	ACCAAGCCT	AGCATT	TATT CGTAAATCCG	1560	
ATGAATTATT	ACATAATGTA	AATGCTGGTA	AATCCACCAC	AAATATCATG	ACTTGATAAT	1620	
GAG						1623	

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 527 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Glu	Leu	Leu	Ile	Leu	Lys	Ala	Asn	Ala	Ile	Thr	Thr	Ile	Leu	Thr
1						5				10			15		
Ala	Val	Thr	Phe	Cys	Phe	Ala	Ser	Gly	Gln	Asn	Ile	Thr	Glu	Glu	Phe
						20			25			30			
Tyr	Gln	Ser	Thr	Cys	Ser	Ala	Val	Ser	Lys	Gly	Tyr	Leu	Ser	Ala	Leu
						35			40			45			
Arg	Thr	Gly	Trp	Tyr	Thr	Ser	Val	Ile	Thr	Ile	Glu	Leu	Ser	Asn	Ile
						50			55			60			
Lys	Glu	Asn	Lys	Cys	Asn	Gly	Thr	Asp	Ala	Lys	Val	Lys	Leu	Ile	Lys
						65			70			75			80

Gln Glu Leu Asp Lys Tyr Lys Asn Ala Val Thr Glu Leu Gln Leu Leu
 85 90 95

Met Gln Ser Thr Pro Pro Thr Asn Asn Arg Ala Arg Arg Glu Leu Pro
 100 105 110

Arg Phe Met Asn Tyr Thr Leu Asn Asn Ala Lys Lys Thr Asn Val Thr
 115 120 125

Leu Ser Lys Lys Arg Lys Arg Arg Phe Leu Gly Phe Leu Leu Gly Val
 130 135 140

Gly Ser Ala Ile Ala Ser Gly Val Ala Val Ser Lys Val Leu His Leu
 145 150 155 160

Glu Gly Glu Val Asn Lys Ile Lys Ser Ala Leu Leu Ser Thr Asn Lys
 165 170 175

Ala Val Val Ser Leu Ser Asn Gly Val Ser Val Leu Thr Ser Lys Val
 180 185 190

Leu Asp Leu Lys Asn Tyr Ile Asp Lys Gln Leu Leu Pro Ile Val Asn
 195 200 205

Lys Gln Ser Cys Ser Ile Ser Asn Ile Glu Thr Val Ile Glu Phe Gln
 210 215 220

Gln Lys Asn Asn Arg Leu Leu Glu Ile Thr Arg Glu Phe Ser Val Asn
 225 230 235 240

Ala Gly Val Thr Thr Pro Val Ser Thr Tyr Met Leu Thr Asn Ser Glu
 245 250 255

Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp Gln Lys Lys
 260 265 270

Leu Met Ser Asn Asn Val Gln Ile Val Arg Gln Gln Ser Tyr Ser Ile
 275 280 285

Met Ser Ile Ile Lys Glu Glu Val Leu Ala Tyr Val Val Gln Leu Pro
 290 295 300

Leu Tyr Gly Val Ile Asp Thr Pro Cys Trp Lys Leu His Thr Ser Pro
 305 310 315 320

Leu Cys Thr Thr Asn Thr Lys Glu Gly Ser Asn Ile Cys Leu Thr Arg
 325 330 335

Thr Asp Arg Gly Trp Tyr Cys Asp Asn Ala Gly Ser Val Ser Phe Phe
 340 345 350

Pro Gln Ala Glu Thr Cys Lys Val Gln Ser Asn Arg Val Phe Cys Asp
 355 360 365

Thr Met Asn Ser Leu Thr Leu Pro Ser Glu Ile Asn Leu Cys Asn Val
 370 375 380

Asp Ile Phe Asn Pro Lys Tyr Asp Cys Lys Ile Met Thr Ser Lys Thr
 385 390 395 400

Asp Val Ser Ser Ser Val Ile Thr Ser Leu Gly Ala Ile Val Ser Cys
 405 410 415

Tyr Gly Lys Thr Lys Cys Thr Ala Ser Asn Lys Asn Arg Gly Ile Ile
420 425 430

Lys Thr Phe Ser Asn Gly Cys Asp Tyr Val Ser Asn Lys Gly Met Asp
435 440 445

Thr Val Ser Val Gly Asn Thr Leu Tyr Tyr Val Asn Lys Gln Glu Gly
450 455 460

Lys Ser Leu Tyr Val Lys Gly Glu Pro Ile Asn Phe Tyr Asp Pro
465 470 475 480

Leu Val Phe Pro Ser Asp Glu Phe Asp Ala Ser Ile Ser Gln Val Asn
485 490 495

Glu Lys Ile Asn Gln Ser Leu Ala Phe Ile Arg Lys Ser Asp Glu Leu
500 505 510

Leu His Asn Val Asn Ala Gly Lys Ser Thr Thr Asn Ile Met Thr
515 520 525

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CATGACTTGA TAATGAG

17

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGAACATTAA CTCCTAG

17

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCCGCGCG CGCG 14

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCGCGCGCGC TTAA 14

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGTTGGTAT ACCAGTGTTA TAACT 25

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/CA 98/00840

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86 C12N15/45 C07K14/115 C07K14/135 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 40945 A (CONNAUGHT LAB ;LI XIAOMAO (CA); EWASYSHYN MARY E (CA); SAMBHARA SU) 19 December 1996 cited in the application see the whole document ---	1-53
Y	LILJESTROEM P. ET AL.: "A NEW GENERATION OF ANIMAL CELL EXPRESSION VECTORS BASED ON THE SEMILOKI FOREST VIRUS REPLICON" BIO/TECHNOLOGY, vol. 9, December 1991, pages 1356-1361, XP000616021 cited in the application see the whole document ---	1-53
A	US 5 614 372 A (LILJA HANS ET AL) 25 March 1997 see column 5, line 61 - line 65 ---	1-53
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "I" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "A" document member of the same patent family

Date of the actual completion of the international search

8 January 1999

Date of mailing of the international search report

22/01/1999

Name and mailing address of the ISA

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NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/00840

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHLESINGER S.: "ALPHAVIRUSES - VECTORS FOR THE EXPRESSION OF HETEROLOGOUS GENES" TRENDS IN BIOTECHNOLOGY, vol. 11, no. 1, 1 January 1993, pages 18-22, XP000358625 see page 20, left-hand column, last paragraph - right-hand column, paragraph 1 ---	1-53
A	WO 96 17072 A (VIAGENE INC) 6 June 1996 see the whole document, especially page 38, lines 20-31 and page 61 lines 12-32 ---	1-53
A	ZHOU X. ET AL.: "Self-replicating Semliki-Forest virus RNA as recombinant vaccine." VACCINE, vol. 12, no. 16, 1994, pages 1510-1514, XP002089524 cited in the application see the whole document -----	1-53

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/00840

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 45-49 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

(Information on patent family members)

International Application No
PCT/CA 98/00840

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9640945 A	19-12-1996	AU	695527 B	13-08-1998
		AU	6117696 A	30-12-1996
		CA	2223610 A	19-12-1996
		EP	0832253 A	01-04-1998
		US	5843913 A	01-12-1998
US 5614372 A	25-03-1997	EP	0811164 A	10-12-1997
		WO	9626442 A	29-08-1996
WO 9617072 A	06-06-1996	AU	4594996 A	19-06-1996
		EP	0797679 A	01-10-1997
		US	5814482 A	29-09-1998
		US	5843723 A	01-12-1998
		US	5789245 A	04-08-1998